Coping with cyclic oxygen availability: evolutionary aspects

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Synopsis Both the gradual rise in atmospheric oxygen over the Proterozoic Eon as well as episodic fluctuations in oxygen over several million-year time spans during the Phanerozoic Era, have arguably exerted strong selective forces on cellular and organismic respiratory specialization and evolution. The rise in atmospheric oxygen, some 2 billion years after the origin of life, dramatically altered cell biology and set the stage for the appearance of multicelluar life forms in the Vendian (Ediacaran) Period of the Neoproterozoic Era. Over much of the Paleozoic, the level of oxygen in the atmosphere was near the present atmospheric level (21%). In the Late Paleozoic, however, there were extended times during which the level of atmospheric oxygen was either markedly lower or markedly higher than 21%. That these Paleozoic shifts in atmospheric oxygen affected the biota is suggested by the correlations between: (1) Reduced oxygen and the occurrences of extinctions, a lowered biodiversity and shifts in phyletic succession, and (2) During hyperoxia, the corresponding occurrence of phenomena such as arthropod gigantism, the origin of insect flight, and the evolution of vertebrate terrestriality. Basic similarities in features of adaptation to hyopoxia, manifest in living organisms at levels ranging from genetic and cellular to physiological and behavioral, suggest the common and early origin of a suite of adaptive mechanisms responsive to fluctuations in ambient oxygen. Comparative integrative approaches addressing the molecular bases of phenotypic adjustments to cyclic oxygen fluctuation provide broad insight into the incremental steps leading to the early evolution of homeostatic respiratory mechanisms and to the specialization of organismic respiratory function.

Prelude

The gradual rise in atmospheric oxygen level and the occurrence of prolonged episodes of atmospheric hypoxia and hyperoxia have both exerted a strong selective effect on molecular, physiological, and behavioral aspects of cellular and organismic respiration (Kasting and Siefert 2002; Webster 2003; Berner et al. 2007; Canfield et al. 2007). The initial accumulation of oxygen in the atmosphere began in the Proterozoic Eon, about 2.3 billion years ago (Fig. 1) and, while atmospheric oxygen has remained near present atmospheric levels (21%) for the past 600–700 million years, there were prolonged episodes of both atmospheric hypoxia and hyperoxia in the Late Paleozoic (Ward et al. 2006; Berner et al. 2007). The evolutionary significance of both the initial rise in oxygen, as well as the Late Paleozoic fluctuations, holds increasing scientific interest, particularly when viewed in the context of the numerous and diverse examples of respiratory adaptations to periodic hypoxia that have been documented for extant species as a result of either changes in activity level or in habitat conditions (Lee et al. 2005; Gerlach et al. 2006; Giomi et al. 2007; Fig. 2).

The present article reports on the proceedings of the Cyclic Oxygen Symposium of the 1st International Congress of Respiratory Biology. The Symposium's objective was to establish a comparative and historical perspective in which to examine the link between the changes in oxygen that have occurred over time (i.e., from the Proterozoic to the present) and the diversity of the molecular, biochemical, physiological, and behavioral responses that characterize the respiratory plasticity of many

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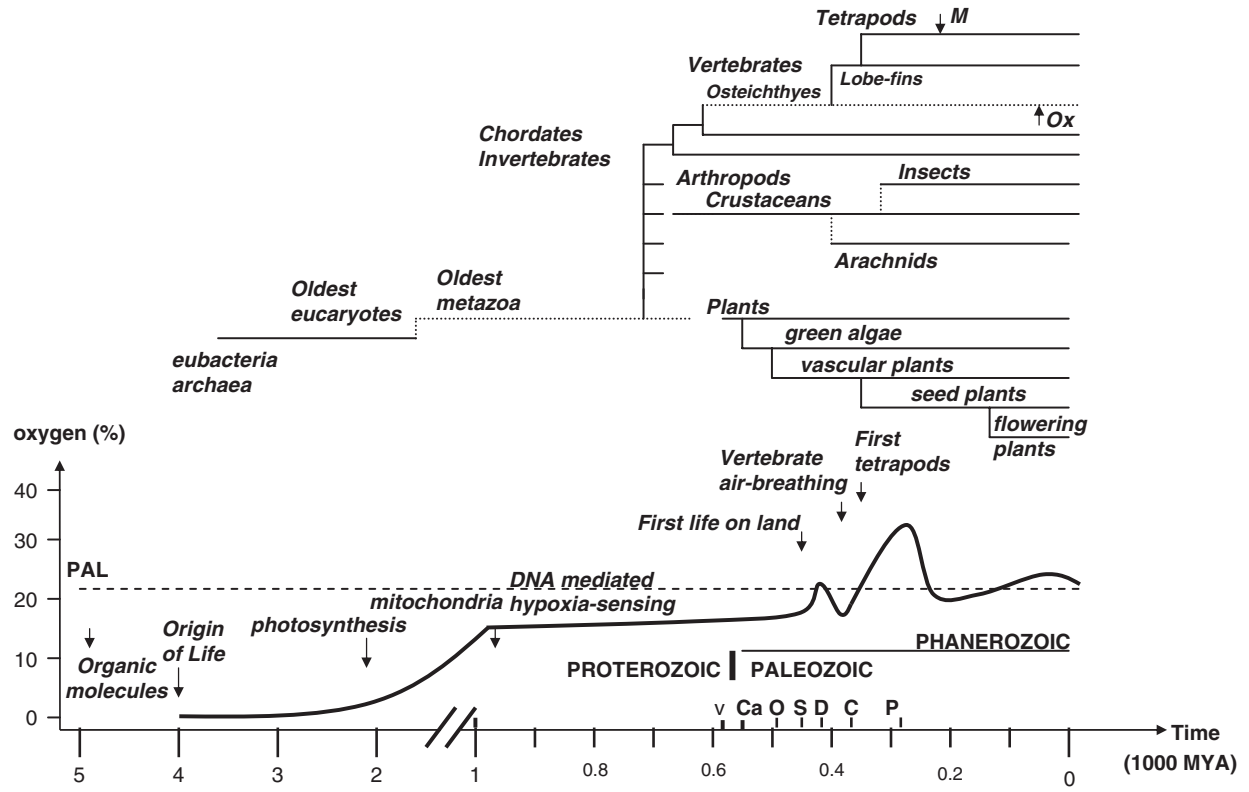

Fig. 1 Relationships between geologic time, atmospheric oxygen concentration, and the phylogenetic history of early life forms, animals, and plants. **Top**: Approximate phylogenetic relationships among taxa addressed in this article. Adapted from Dudley (1998), Lang et al. (1999), Webster (2003), Ward et al. (2006) and Berner at al. (2007). Ox = the mudskipper subfamily Oxudercinae; M = Mammals. **Bottom**: Qualitative representation of atmospheric oxygen concentration (black tracing) in relation to evolutionary milestones in respiratory processes (arrows). PAL is the present atmospheric level of oxygen (21%). Geologic time abbreviations: V = Vendian Period of the Proterozoic Era; Paleozoic Era periods are Ca = Cambrian; O = Ordivician; S = Silurian; D = Devonian; C = Carboniferous; P = Permian. Note that the fossil record of most animal and plant groups does not begin before the Cambrian and that the geologic time scale is in 1000 million years ago (mya).

extant species. In providing details about the respiratory plasticity of extant species, this work seeks to demonstrate the influences of habitat on respiration and also demonstrates the thread of continuity for respiratory specialization that extends from deep time to the present.

Oxygen-dependent evolution

The increase in atmospheric oxygen during the Proterozoic Eon remarkably affected the complexity and specialization of life forms and metabolic processes (Fig. 1). Life originated and persisted for over 2 billion years in a strictly anaerobic, reducing environment (Kasting and Siefert 2002; Canfield et al. 2007). The primitive life forms of this environment, however, were dramatically altered by the evolution of photosynthesis and gradual accumulation of oxygen in the atmosphere. Early forms of hypoxia-sensitive gene expression appear related to detecting pockets of hypoxia in a predominantly oxidative habitat and to the development of metabolic pathways producing antioxidants (Fig. 1) (Webster 2003). The discrete organelle structure of eukaryotic cells is hypothesized to have resulted from strong forces, driven by increased ambient oxygen, for selection of the symbiotic ''fusion'' of primitive cells and respiratory bacteria (Lang et al. 1999). A characteristic of eukaryotic cells is the requirement for regional differences in intracellular redox potential: delicate DNA molecules in the nucleus are, in this manner, sequestered from the oxidative stress of electron transport and high-energy transduction in the mitochondria. This compartmentalization after the explosion of oxygen pressure, therefore, reflects the selective advantage of separating oxidative reactions from the cell body with a strict redox requirement (Webster 2003).

The gradual accumulation of atmospheric oxygen helped to set the stage for the dramatic explosion in biodiversity that began in the Vendian (Ediacaran) Period of the late (Neo) Proterozoic and extended

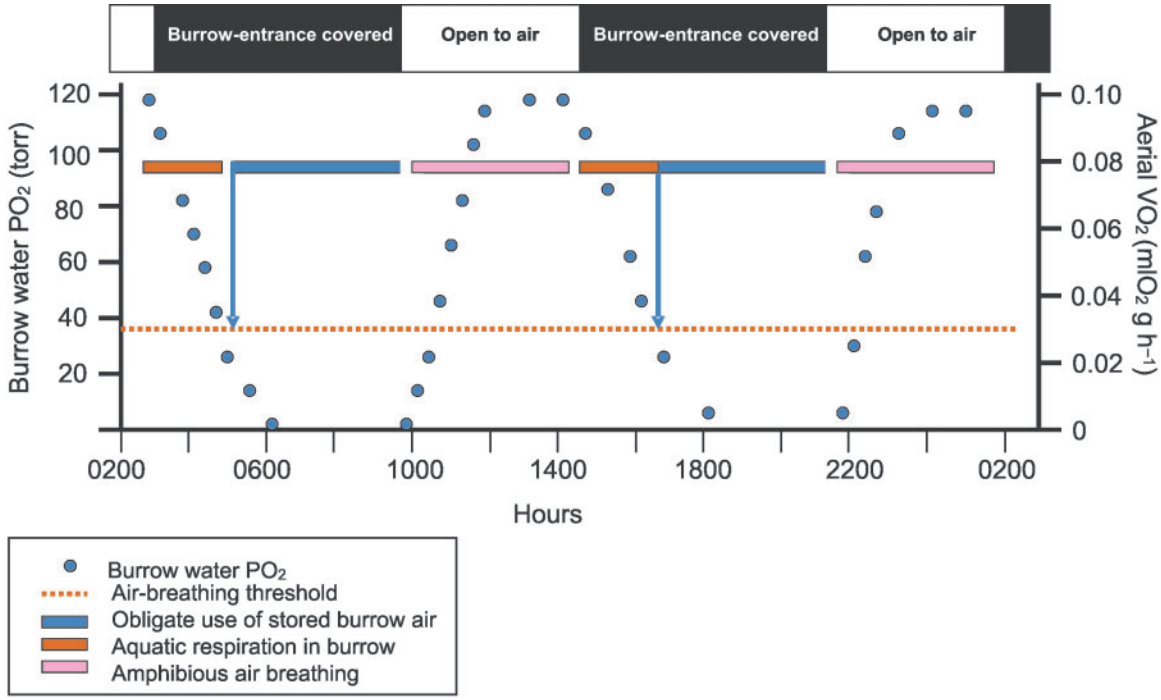

Fig. 2 Integrated model for the daily pattern of tidal influence on the O_2 levels in burrows on mudflats and its effect on the respiratory mode and rate (red, blue, and pink horizontal bars) of the mudskipper *Scartelaos histophorus*. Horizontal bar across the top shows times when the rising tide covers the burrow entrance (black) and when, on the falling tide, the entrance is open to air (white). Blue dots show changes in burrow-water PO_2, which is highest at the time the tide reaches the entrance and falls during high tide (i.e., burrow water is flushed by a falling tide but stagnant during high tide). Respiratory mode of fish is dictated by its behavior and by the tidal cycle. During low tide the fish uses amphibious air-breathing (pink bar), while it is active on the surface of the mudflat (average $VO_2 \sim 0.08$ ml $g.h^{-1}$ at 25°C). As the rising tide reaches the burrow entrance, the fish retreats into its burrow where it remains until the burrow is again exposed by the retreating tide. In the early part of confinement, the water in the burrow contains sufficient O_2 to permit aquatic respiration (red bar). Also, the fish would have access to well-oxygenated water at the opening of the burrow. However, the high biological O_2 demand of the burrow water rapidly reduces PO_2 to the air-breathing threshold (horizontal, red dashed line), which requires the fish to respire aerially (blue arrow and bar) using the air it stored in the burrow during high tide. Modified from Lee et al. (2005).

into the Paleozoic Era (Cambrian Period) (Fig. 1). Another effect of oxygen on evolution played out in the Late Paleozoic with the occurrence of prolonged, episodic time spans during which atmospheric oxygen levels either dropped markedly below, or increased dramatically above, the present atmospheric level of 21% (Fig. 1). Examination of the fossil record in relation to these atmospheric oxygen shifts shows correlations between hyperoxia and the transformation and radiation of certain groups, and between hypoxia and reduced biodiversity and extinction (Fig. 1) (Graham et al. 1995; Dudley 1998; Ward et al. 2006; Berner et al. 2007). The pulse increase in atmospheric oxygen during the Late Paleozoic appears to have had a metabolic effect in that it correlates with the appearance of arthropod gigantism, the origin of insect flight, and invasion of land by vertebrates and the subsequent radiation of the tetrapods (Fig. 1) (Graham et al. 1995; Graham 1997; Dudley 1998; Graham and Lee 2004; Clack 2005).

Evolution of oxygen-sensing

A molecular-based response to reduced oxygen availability appears to be encoded in the genome of virtually all organisms and thus argues for both a strong selective influence and the evolutionary heritage of access to oxygen (Wang et al. 1995; Webster 2003).

The capacity of eukaryotes to switch from oxidative metabolism to glycolysis by shutting down mitochondrial metabolism in hypoxia, is a consequence of selection for gene-mediated regulation of the ancient cellular processes combined by symbiotic fusion (Webster 2003). Molecular dissection of the coordinated regulation of glycolytic enzymes in eukaryotic cells demonstrates that the positive control of glycolytic genes by hypoxia

is mediated by hypoxia-responsive genomic DNA elements (HREs; Wang et al. 1995; Webster 2003). For prokaryotes, molecular genetic studies suggest that this major *cis*-acting regulatory paradigm can be traced back to ancestral redox-sensing DNA sites (Webster 2003). Analyses of the primitive sequences suggest that mutagenic events, possibly driven by oxygen in the Proteorozoic Eon (Fig. 1), led to the oxygen-sensing pathway that now exists in eukaryotes (Webster 2003). Thus, HREs in glycolytic-enzyme genes along with their current oxygen-sensing properties may be ancient, having first appeared in the earliest eukaryotic cells.

Identification of the *trans*-acting factors and oxygen sensors that control HRE-dependent gene activity under hypoxia was achieved by intense studies in the 1990s (Wang et al. 1995). Hypoxia-inducible factor 1 (HIF-1) is the *trans*-acting moiety that binds to the HRE and activates the transcription of glycolytic enzyme and other HRE-containing genes (Wang et al. 1995). The HIF-1α subunit of HIF-1 is stabilized by low oxygen due to the oxygen-dependent hydroxylation of proline residues that determine whether or not the protein is ubiquinated and degraded by the proteosome. Nonubiquinated protein accumulates under hypoxia and activates all HRE-regulated genes.

The precise molecular mechanisms of oxygen-sensing are not fully defined and some aspects still remain controversial. For example, phagocytic NADPH oxidase is a membrane-bound complex that is sensitive to oxygen tension. Hypoxia affects oxidase function via decrease in substrate (O_2) availability, resulting in reduced production of O_2-reactive intermediates including H_2O_2, and the redox modulation of downstream effectors such as the K+channel in airway chemoreceptors (Fu et al. 2000).

Hypoxia-induced gene expression

In animal models, the functional relevance of the HIF-1-mediated molecular orchestration of the response to hypoxia is well illustrated by the metabolic tuning of striated muscle tissue associated with physiological challenges. During contractile work muscle tissue is frequently subjected to hypoxia as low as 5 Torr (Richardson et al. 1995). After 45 minutes of knee-extensor exercise, the human *vastus lateralis* muscle has elevated levels of HIF-1α protein (Ameln et al. 2005). However, noncontracting skeletal muscle exposed to only 40 Torr of hypoxia will also activate the HIF-1 pathway (Webster 2003; Richardson et al. 2006). Thus, HIF-1 mediated hypoxia-signaling represents a possible key regulatory pathway responsible for the shift in muscle metabolism in hypoxia towards a higher reliance on carbohydrates (Boning 1997; Ngumbela et al. 2003; Fluck 2006).

The down-regulation of transcripts of fatty-acid metabolism by hypoxia broadens the regulatory scope of oxygen-responsive genes to that of general metabolic control (Dapp et al. 2006). The coupling of respiratory and glycolytic-enzyme mRNA expression in muscle and the negative feedback to fatty-acid metabolism is consistent with that seen in muscle-specific HIF-1α knockout mice that also display increased fatty-acid oxidation (Mason et al. 2004). This phenomenon is also seen in hypoxic cardiac myocytes in which factors such as the transcriptional regulator of fatty-acid metabolism, Peroxisome proliferator-activated receptor-alpha (PPARα), may be important as well (Ngumbela et al. 2003). However, the hypoxia-induced expression signature of metabolic transcript expression is reversed in HIF-1α—heterozygous muscle. This argues for a key role of HIF-1α in the maximization of metabolic efficiency under oxygenation constraints and implies a larger involvement of HIF-1α in oxygen-dependent *trans*-metabolic control via gene expression in muscle tissue.

Genetic approaches to understanding the physiology of hypoxic and ischemic muscle were long hampered by the embryonic lethality of HIF-1α gene ablation and by the absence of pharmacological interventions that modulated HIF-1 activity in intact tissue. Recent work circumvented these limitations by measuring transcriptional changes caused by a strenuous-exercise hypoxic stimulus on viable HIF-1α heterozygous-deficient mice (Dapp et al. 2006). Following 24 h exposure to a simulated elevation of 6000 m, *soleus* muscle transcripts from these mice showed that coordinated induction of glycolytic-gene expression had taken place (Webster 2003; Dapp et al. 2006). These studies also indicated that mitochondrial biogenesis is induced by episodes of hypoxia (Hoppeler et al. 2003). Similarly, HIF-1α responses in the different organs of the carp, an hypoxia-tolerant fish (Rissanen et al. 2006) indicate the broad distribution of this ancient molecular switch in counter-control of oxygen-dependent processes in vertebrates. To date cellular oxygen receptors and the induction and expression of the HIF-1α transition factor have both been well documented for fishes (Gracey et al. 2001; Soitamo et al. 2001; Nikinmaa and Rees 2005; Rissannen et al. 2006).

Further, in this regard, we note recent evidence from humans supporting the claim for a molecular mediation of the selective pressure of oxygen (Gelfi et al. 2004; Malacrida et al. 2007).

Phenotypic traits related to cyclic oxygen fluctuations

Other comparative animal studies address the molecular bases of phenotypic adjustments to cyclic oxygen fluctuation within the context of differences in habitat and activity level.

Some terrestrial invertebrates confront hypoxic and even anoxic challenges in their environments and in the course of their energy-consuming activities. Among arthropods (Fig. 1), variation in both hypoxia and anoxia tolerance relates to differences in the respiratory system and is also likely affected by microhabitat conditions.

Air-breathing crustaceans and arachnids are less likely to encounter hypoxic environments than are insects and myriapods and respirometric comparisons show that the former two are correspondingly less tolerant of both hypoxia and anoxia (Schmitz and Harrison 2004). Arthropods that have a tracheal system, especially those that are capable of tracheal ventilation (many insects), have a lower critical oxygen pressure (i.e., the pressure that triggers a reduction in oxygen uptake, anerobic metabolism, or both) than do arthropods lacking tracheae. The tracheal system also provides some insects with an extraordinary capacity to recover from anoxia via passive diffusion (Schmitz and Harrison 2004). Moreover, the insect tracheal system is plastic and increases dimension with long-term hypoxia during development (Ghabrial et al. 2003). Exercise-induced aerobic-performance tests with spiders also demonstrate the advantage of a specialization towards a ventilated tracheal system during activity (Schmitz 2005).

Some aquatic crustaceans, however, appear exceptional in this regard as they constitute an example of extreme physiological adaptability to cyclic and stochastic environmental perturbations. The plasticity of their respiratory system, with particular reference to the modulation of oxygen transport, allows crustaceans to sustain selective pressures of extremely variable environments (Bridges 2001). Recent biochemical investigations on ''respiratory plasticity'' in portunid crabs, imply a distinct respiratory role for the oxygen-transporting molecule hemocyanin (Giomi et al. 2007). Subunit changes in this copper-containing respiratory pigment have been previously linked to alterations in oxygen transport and to gene regulation (Terwilliger and Dumler 2001). Also, electrophoretic and chromatographic data for the crabs *Carcinus aestuarii* and *Liocarcinus depurator* in the North Adriatic sea demonstrate marked, population-level differences in the composition of hemocyanin subunits and these in turn correlate with seasonal effects on, and regional differences in, environmental oxygen level and thus point to the plasticity of respiratory responses that may occur among distinct populations (Giomi et al. 2007). Finally, experimental emersion of the semi-terrestrial species *Carcinus aestuarii* reveals the integration of molecular traits such as the allosteric modulation of hemocyanin for air breathing and morphological features such as gill structure (Giomi et al. 2007). In this case reduced, oxygen availability seems to constitute a ''driving force to emerge'' for intertidal crustaceans, which are favored by maintaining aerobic metabolism and activity out of water, as opposed to the option of switching to anaerobiosis and a quiescent state while submerged in hypoxic water.

With regard to the molecular underpinnings of the responses of terrestrial arthropods to hypoxia, recent work on the regulatory pathways of morphological and biochemical responses to hypoxia indicate that some oxygen-related mechanisms such as HIF-1α, discovered in higher and lower vertebrates, are also expressed in insects (Schmitz and Harrison 2004; Ameln et al. 2005). A survey of the literature, however, suggests that the modulation of oxygen-carrier proteins is normally not involved in the hypoxic maintenance of terrestrial arthropod gas exchange (Schmitz and Harrison 2004).

Vertebrates evolved in water and the first air-breathing vertebrates were fishes living in lowland environments subject to drought and hypoxia (Graham 1997; Graham and Lee 2004). One lineage of the bony fishes (Class Osteichthyes), the Sarcopterygii, had limb-like or ''lobed fins'' as well as lungs and it was from this group that the first tetrapods evolved and invaded land during the Late Devonian Period of the Paleozoic Era (Fig. 1) (Graham 1997; Graham and Lee 2004; Clack 2005). The ancestral relationship of Paleozoic lobefins to the tetrapods is well documented by limb structure, the presence of a lung, and many other features, and the earliest tetrapods are ancestral to all other terrestrial vertebrates, including mammals (Fig. 1) (Graham 1997; Dudley 1998; Graham and Lee 2004; Clack 2005; Ward et al. 2006). While the origin of vertebrate terrestriality appears linked to diverse environmental and ecological factors (Graham 1997; Graham and Lee 2004; Clack 2005),

the fossil record shows that, once on land, the radiation of the early tetrapods occurred during a period of atmospheric hyperoxia in the Late Paleozoic (Fig. 1) (Dudley 1998; Graham and Lee 2004; Clack 2005; Ward et al. 2006).

With respect to air breathing among fishes, the 300 million year history of fish evolution since the Paleozoic has seen the persistence of some air-breathing fish taxa as well as the independent origin, among many fish groups, of air breathing, novel air-breathing organs distinct from the lung, and amphibious behavior (Graham 1997; Graham and Lee 2004). While only remotely related to the lobefin ancestors of tetrapods, these extant air-breathing fishes stand as proxy models for the Paleozoic evolution of vertebrate aerial respiration and terrestriality. Moreover, among both air-breathing and non-air-breathing fishes there is ample documentation for the role of hypoxia in the induction of a suite of phenotypic changes that alter hemoglobin-oxygen affinity, respiration, and metabolism (Gracey et al. 2001; Graham and Lee 2004; Nikinmaa and Rees 2005).

One group of modern teleosts in which the characters of air breathing and terrestriality are well developed, is the mudskippers (family Gobiidae, subfamily Oxudercinae), a group that likely first appeared in the early Cenozoic Era (about 40–50 mya) (Fig. 1). Mudskippers live on mudflats, they breathe in air and water, are active on land, and have a specialized behavior for the storage of air in their mudflat burrows. This air is important for respiration during high tide when mudskippers such as *Scartelaos histophorus* are confined to burrows that are filled with hypoxic water. The occurrence of hypoxia in the burrow follows a daily tidal pattern and requires that the fish switch from aquatic to aerial respiration, exploiting the air deposited in the burrow during low tide (Fig. 2) (Lee et al. 2005). While *Scartelaos histophorus* breathes its stored air during each confinement in the burrow, other mudskipper species rear their eggs in the air-containing pockets they construct and maintain within their burrows. Experiments demonstrate that, in addition to breathing the stored air, some mudskippers can sense the oxygen level in this air pocket and will replace hypoxic air with fresh air, which they transport into the burrow by means of mouth gulps (Graham and Lee 2004; Lee et al 2005).

As in fishes, certain mammals, also show plasticity in respiratory proteins in response to hypoxic conditions. The mole rat, *Spalax ehrenbergi*, a burrow-dwelling species that naturally encounters hypoxia in its habitat, has correspondingly higher levels of cytoglobin and neuroglobin than occur in the common rat. These proteins appear important in either elevated oxygen-transport or ROS (reactive oxygen species) scavenging under extreme hypoxia (Gerlach et al. 2006). In summary, as in arthropods, there is among the vertebrates the thread of continuity for HIF-1α-mediated responses to hypoxia that extends from fishes to mammals. This trait can be especially elaborated among certain specialized air breathers that, because of environmental factors, experience cyclic fluctuations in their access to oxygen.

Conclusion

The Cyclic Oxygen Symposium of the 1st International Congress of Respiratory Biology documented the extent of respiratory plasticity among different species and also demonstrated the thread of a common molecular basis for phenotypic responses to altered oxygen availability that extends back into the Proterozoic Eon. Comparative studies with a diversity of animal taxa show a broad range of respiratory adaptations as well as integrated responses to hypoxia that utilize biochemical, physiological, and behavioral mechanisms to either sustain oxygen delivery or induce a reduction in oxygen-mediated energy metabolism. Recent molecular findings on the regulatory modes of the response to hypoxia support the validity of comparative approaches in identifying and predicting metabolic adjustments (Vogt et al 2001). Hypoxia-regulated gene expression very likely reflects an ancient adaptive trait that was established in primitive eukaryotes in response to alterations in levels of oxygen. In more derived animal groups, an hypoxia-induced selection has added adaptive layers to respiratory function in the form of facultative anaerobiosis, augmentation of oxygen-extraction and transport capacity, and even novel respiratory mechanisms (e.g., air breathing). Among air-breathing fishes, for example, subtle environmental differences as well as oxygen level, may account for dramatic differences in the extent of development of organs used in air-breathing (Graham 1997). The range of air-breathing animal models (e.g., arthropods, vertebrates, and others) together with an emerging array of novel biotechnological tools offer the possibility for future comparative investigations that will expand understanding of the molecular nature of respiratory plasticity and its evolutionary significance (Pennisi 2002; Grus and Zhang 2006; Harrison et al. 2006). In this regard a key requirement will be to distinguish between ancient

molecular adaptations dating back to the oxygen-responses of Protereozoic prokaryotes, those by the common ancestors of the animal phyla that appeared in the Neoproterozoic (Vendian) (Fig. 1) and those that have resulted from the evolution and radiation of different groups over Phanerozoic time.

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References

Ameln H, Gustafsson T, Sundberg CJ, Okamoto K, Jansson E, Poellinger L, Makino Y. 2005. Physiological activation of hypoxia inducible factor-1 in human skeletal muscle. FASEB J 19:1009–11.

Berner RA, Vandenbrooks JM, Ward PD. 2007. Oxygen and evolution. Science 316:557–8.

Boning D. 1997. Altitude and hypoxia training-a short review. Int J Sports Med 18:565–70.

Bridges CR. 2001. Modulation of haemocyanin oxygen affinity: properties and physiological implications in a changing world. J Exp Biol 204:1021–32.

Canfield DE, Poulton SW, Narbonne GM. 2007. Late-Neoproterozoic deep-ocean oxygenation and the rise of animal life. Science 315:92–4.

Clack JA. 2005. Getting a leg up on land. Sci Am 293:100–7.

Dapp C, Gassmann M, Hoppeler H, Fluck M. 2006. Hypoxia-induced gene activity in disused oxidative muscle. Adv Exp Med Biol 588:171–88.

Dudley R. 1998. Atmospheric oxygen, giant Paleozoic insects and the evolution of aerial locomotor performance. J Exp Biol 201:1043–50.

Fluck M. 2006. Functional, structural and molecular plasticity of mammalian skeletal muscle in response to exercise stimuli. J Exp Biol 209:2239–48.

Fu XW, Wang D, Nurse CA, Dinauer MC, Cutz E. 2000. NADPH oxidase is an O_2 sensor in airway chemoreceptors: evidence from K+ current modulation in wild-type and oxidase-deficient mice. Proc Natl Acad Sci USA 97:4374–9.

Gelfi C, De Palma S, Ripamonti M, Eberini I, Wait R, Bajracharya A, Marconi C, Schneider A, Hoppeler H, Cerretelli P. 2004. New aspects of altitude adaptation in Tibetans: a proteomic approach. FASEB J 18:612–4.

Gerlach F, Avivi A, Burmester T, Nevo E, Hankeln T. 2006. Regulation of neuroglobin, cytoglobin and myoglobin in a hypoxia-tolerant mammal, the subterranean mole rat *Spalax ehrenbergi*. Proceedings of the 1st International Congress of Respiratory Biology, P20, p 19.

Ghabrial A, Luschnig S, Metzstein MM, Krasnow MA. 2003. Branching morphogenesis of the Drosophila tracheal system. Annu Rev Cell Dev Biol 19:623–47.

Giomi F, Raicevich S, Ferrarese A, Pranovi F, Di Muro P, Beltramini M. 2007. Structural and functional heterogeneity of hemocyanin: intra- and inter-specific comparison in four species of portunid crabs (Crustacea: Portunidae). Mar Biol 151:1237–47.

Gracey AY, Troll JV, Somero GN. 2001. Hypoxia-induced gene expression profiling in the euryoxic fish Gillichthys mirabilis. Proc Natl Acad Sci USA 98:1993–1998.

Graham JB. 1997. Air breathing fishes: evolution, diversity and adaptation. San Diego, CA: Academic Press.

Graham JB, Dudley R, Aguilar NM, Gans C. 1995. Implications of the late Palaeozoic oxygen pulse for physiology and evolution. Nature 375:117–20.

Graham JB, Lee HJ. 2004. Breathing air in air: in what ways might extant amphibious fish biology relate to prevailing concepts about early tetrapods, the evolution of vertebrate air breathing, and the vertebrate land transition? Physiol Biochem Zool 77:720–31.

Grus WE, Zhang J. 2006. Origin and evolution of the vertebrate vomeronasal system viewed through system-specific genes. Bioessays 28:709–18.

Harrison J, Frazier MR, Henry JR, Kaiser A, Klok CJ, Rascon B. 2006. Responses of terrestrial insects to hypoxia or hyperoxia. Respir Physiol Neurobiol 154:4–17.

Hoppeler H, Vogt M, Weibel ER, Fluck M. 2003. Response of skeletal muscle mitochondria to hypoxia. Exp Physiol 88:109–19.

Kasting JF, Siefert JL. 2002. Life and the evolution of earth's atmosphere. Science 296:1066–68.

Lang BF, Gray MW, Burger G. 1999. Mitochondrial genome evolution and the origin of eukaryotes. Annu Rev Genet 33:351–97.

Lee HJ, Martinez CA, Hertzberg KJ, Hamilton AL, Graham JB. 2005. Burrow air phase maintenance and respiration by the mudskipper *Scartelaos histophorus* (Gobiidae: Oxudercinae). J Exp Biol 208:169–77.

Malacrida S, Katsuyama Y, Droma Y, Basnyat B, Angelini C, Ota M, Danieli GA. 2007. Association between human polymorphic DNA markers and hypoxia adaptation in Sherpa detected by a preliminary genome scan. Ann Hum Gene 1:March 15, 2007 [Epub ahead of print].

Mason SD, et al. 2004. Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. PLoS Biol 2:e288.

Nikinmaa M, Rees BB. 2005. Oxygen-dependent gene expression in fishes. Amer J Physiol Regulatory Interative Comp Physiology 288:1079–90.

Ngumbela KC, Sack MN, Essop MF. 2003. Counter-regulatory effects of incremental hypoxia on the transcription of a cardiac fatty acid oxidation enzyme-encoding gene. Mol Cell Biochem 250:151–8.

Pennisi E. 2002. Comparative physiology. Recharged field's rallying cry: gene chips for all organisms. Science 297:1985–87.

Richardson RS, Duteil S, Wary C, Wray DW, Hoff J, Carlier PG. 2006. Human skeletal muscle intracellular

oxygenation: the impact of ambient oxygen availability. J Physiol 571:415–24.

Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD. 1995. Myoglobin O_2 desaturation during exercise. Evidence of limited O_2 transport. J Clin Invest 96:1916–26.

Rissanen E, Tranberg HK, Sollid J, Nilsson GE, Nikinmaa M. 2006. Temperature regulates hypoxia-inducible factor-1 (HIF-1) in a poikilothermic vertebrate, crucian carp (*Carassius carassius*). J Exp Biol 209:994–1003.

Soitamo AJ, Rabergh CMI, Gassmann M, Sistonen L. 2001. Characterization of a hypoxia-inducible factor (HIF-1α) from rainbow trout. J Biol Chem 276:19699–705.

Schmitz A, Harrison JF. 2004. Hypoxic tolerance in air-breathing invertebrates. Respir Physiol Neurobiol 141:229–42.

Schmitz A. 2005. Spiders on a treadmill: influence of running activity on metabolic rates in *Pardosa lugubris* (Arachnida, Araneae, Lycosidae) and *Marpissa muscosa* (Arachnida, Araneae, Salticidae). J Exp Biol 208:1401–11.

Terwilliger N, Dumler K. 2001. Ontogeny of decapod crustacean hemocyanin: effects of temperature and nutrition. J Exp Biol 204:1013–20.

Vogt M, Puntschart A, Geiser J, Zuleger C, Billeter R, Hoppeler H. 2001. Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. J Appl Physiol 91:173–82.

Ward PD, Labanderia C, Laurin M, Berner RA. 2006. Confirmation of Romer's Gap as a low oxygen interval constraining the timing of initial arthropod and vertebrate terrestrialization. Proc Nat Acad Sci USA 103:16818–22.

Wang GL, Jiang BH, Rue EA, Semenza GL. 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci USA 92:5510–4.

Webster KA. 2003. Evolution of the coordinate regulation of glycolytic enzyme genes by hypoxia. J Exp Biol 206:2911–22.